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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Thomas C. Terwilliger	Docket No :	S-91,732
Serial No.:	09/512,962	Examiner:	Hartter
Filed :	February 25, 2000	Art Unit:	1631
For :	LIKELIHOOD-BASED MODIFICATION OF EXPERIMENTAL CRYSTAL STRUCTURE ELECTRON DENSITY MAPS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

Sir.

I, Li-Wei Hung, hereby state:

1. I am a staff member of the Biological and Quantum Physics Group at the Los Alamos National Laboratory, with a B.S. in physics from National Taiwan University and a PhD in Biophysics from the University of California, Berkley. I have 5 years teaching experience in teaching undergraduate physics and biophysical chemistry and more than 10 years research experience in protein crystallography.

2. I consider myself to be a person of ordinary skill in the art of protein crystallography.

3. In an action from the Patent and Trademark Office dated May 20, 2003, it is stated at several locations that "the electron densities of proteins is a complex concept with well known complexities of electron orbital theory and/or quantum mechanical representation of electron wave/particle characteristics . . . ." While this may be true if extremely accurate probabilistic distribution of individual electrons is desired, it is not relevant to the present invention. The invention described in the subject patent application applies well-known mathematical analytical techniques to improve experimental information that is first obtained using only well known techniques in X-ray crystallography applied to an unknown protein structure. The experimental data is first obtained as a diffraction pattern of scattered photons from the application of X-rays to the unknown protein structure, represented as a 3-dimensional matrix whose

components correspond to the diffraction intensities at the reciprocal lattice points. These results and the analysis thereof require no knowledge of electron orbital theory and/or quantum mechanics.

4. The foundation of the theory of X-ray crystallography, Bragg's Law, is a description of 3-dimensional coherent scattering based on Young's model in classical physics. The relationship between the diffracting object (protein structure) and the diffraction pattern is a simple Fourier transformation (FT). Using techniques that are conventional and well known to persons skilled in protein crystallography, the 3-dimensional matrix of photon intensities is converted to structure factors (pairs of intensities and phases). Electron densities are then calculated from a FT of the set of structure factors and are represented by a three dimensional array with numerical values of electron density at each grid point in the 3-dimensional matrix. The mathematical procedures described in the subject patent application involve only the linear algebra of 3-dimensional arrays that are formed using conventional X-ray crystallographic techniques for protein structures that are well known to persons skilled in protein crystallography.

5. I have reviewed the remarks in the office action and consider that the process described in the subject patent application could be performed by a person of ordinary skill in protein crystallography without any undue experimentation and using standard techniques taught in upper division undergraduate mathematical physics courses that would be in the curriculum of persons skilled in protein crystallography. I have the following specific statements:

a Histograms of electron density over the "electron density map" as represented by the 3-dimensional matrix described above are readily formed from the matrix as described in the patent application. Construction of a histogram is fully described at page 15, lines 15-22, and simply involves dividing the electron density map into protein and solvent regions using the criteria set forth and then determining the number of points with each electron density in the electron density map and normalizing the result. This is a straightforward procedure that is well within the skill of a person skilled in protein crystallography.

b Claim 10, steps c. and f., require fitting probability distribution functions to the histograms formed from the electron density maps for a model map and for an experimental map. The probability distribution functions set out in the patent application are Gaussian functions and the fitting of data to a Gaussian function using a least squares fitting technique is taught at the undergraduate level in university science and engineering courses. There are numerous software

techniques available to perform this procedure. A typical crystallographic electron density map contains about 1,000,000 grid points with values representing the electron densities at the corresponding position. A histogram is generated from these numeric values sufficient to oversample the Gaussian curve, where the height of each histogram value represents a data point on the Gaussian curve. This is explained in the patent application at pages 14-16 in sufficient detail to permit a person skilled in protein crystallography to determine the fitting coefficients from the histograms.

c. Claim 14 requires evaluating the terms of a Taylor's series expansion of the log-likelihood of the experimental electron density map using a Fast Fourier Transform (FFT). The FFT is a standard computational technique to rapidly calculate a discrete Fourier transformation with periodical boundary conditions and is a taught in standard upper division and graduate courses of universities. A standard Fortran FFT library for crystallographic purposes has been available for thirty years (see Ten Eyck, Acta Cryst., A29, pg. 486 (1973) and the Ten Eyck publication in the patent application) and is well known to persons skilled in protein crystallography. The tasks related to generating protein electron density maps with FFT described in the patent application can be done with any one of several packages well known to protein crystallographers

d. Claim 10, steps b and e., require that histograms be formed over identified protein and solvent regions of the appropriate electron density map. Separation of an electron density map into protein and solvent regions is shown at page 12, lines 8-14, and page 15, lines 15-18, of the patent application, where all points in the electron density map with values above a threshold set by the overall solvent content of the protein crystal (step e) or within a specified distance of an atom in the model (step b) are designated "protein" and the remaining points are designated "solvent". It should be noted that Equation 17 at page 12 of the patent application is the initial statement of the log likelihood function that incorporates information from the protein and solvent regions in an overall log-likelihood statement. The remaining portions of the patent application are directed to a practical approach to solving Equation 17.

e. Claim 10, step g., requires that the overall experimental log-likelihood of the electron density in the protein and solvent regions of the experimental map be determined from the experimental probability distribution function. The patent application at page 19, lines 1-4 sets out the procedure for obtaining this log-likelihood by determining the probability of the electron density at each point in the

protein or solvent regions of the electron density map from Equation 22, which is simply a Gaussian formula with values from least squares fitting as discussed above. The overall log-likelihood of the electron density is then the sum of the logarithms of the densities determined in each region using Equation 22. This calculation is straightforward and is well within the capability of a person skilled in protein crystallography.

6. Based on the above facts, it is my conclusion that the description of the invention set out in the patent application is in sufficient detail for a person skilled in protein crystallography to carry out the steps set out in Claims 10-14 without any experimentation other than obtaining conventional X-ray diffraction data from a protein crystal where features of resulting experimental electron density maps are processed using well-known mathematical techniques to obtain a revised electron density map that is believed to better represent the actual physical structure of a protein.

All statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Li-Wei Hung

September 29, 2003
Date